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(54) Title: IMPLANTATION COMPOSITION COMPRISING GLASS PARTICLES

(57) Abstract

There is provided a composition suitable for implantation in soft tissue (for example at or around a body orifice) in order to augment the volume of soft tissue. The composition described comprises particles of a, preferably water-soluble, biodegradable glass in a suitable carrier medium, such as glycerol. The particles, which are desirably irregularly shaped, may have an average particle diameter of from 50 μm to 2000 μm, preferably 50 μm to 300 μm. By injecting the particles into soft tissue, for example the bladder submucosa, it is possible to bulk up the soft tissue where this is required. This procedure can be applied to treat conditions such as vesicoureteric reflux. Additionally the procedure could be used cosmetically.

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IMPLANTATION COMPOSITION COMPRISING GLASS PARTICLES

The present invention is concerned with a composition suitable for implantation at or in the vicinity of a body orifice or sphincter muscle to aid correct function.

Many body functions rely upon the correct functioning of sphincter muscles. For example, the pyloric sphincter controls when the contents of the stomach pass into the small intestine. Similarly, the urethral sphincter controls when the contents of the bladder are voided. Incorrect functioning due to premature relaxation of such sphincter muscles can be problematic, and in the case of stress urinary incontinence (malfunction of the urethral sphincter) highly distressing to the patient.

Premature relaxation of a sphincter muscle often occurs when the sphincter muscle itself lacks sufficient bulk to adequately close the orifice in question. One option to overcome the problem is by implanting bulking material in the submucosa surrounding the orifice, thereby reducing the area to be closed by the sphincter muscle. Generally, the bulking material is injected

into the site to augment the soft tissue present. Suitable bulking materials are available commercially and are generally in the form of spherical particles or beads based on silicone, PTFE or collagen. These beads are suspended in a carrier fluid such as glycerine or hydrogel. The carrier fluid acts as a lubricant during the implantation process and assists expulsion of the implant from the syringe through an endoscopic needle. The carrier fluid is eliminated from the body and the implant material gradually becomes encapsulated by collagen at the implant site. The collagen capsule which forms around the implanted material adds to the bulk at the site. One such bulking material is MACROPLASTIQUE (Trade Mark) of Uroplasty, Inc.

Existing implants do not biodegrade but remain permanently in the body of the patient. Recently, concern has been raised that such implants may gradually migrate away from the site of implantation during the lifetime of the patient. Thus, the original problem may recur as the size of the implant gradually decreases due to migration of the beads inserted. The patient will therefore need to undergo a further procedure in order to insert more beads at the site concerned. The migrating implant may, in addition, cause irritation and such implants have been reported to be associated with cancer, auto-immune and connective tissue disease.

In addition to stress urinary incontinence, such implants have also been used to prevent vesicoureteral reflux. Vesicoureteric reflux is a condition occurring in babies and small children where the ureteral orifice is incompletely closed during contraction of the bladder. Urine is thus allowed to reflux back up the ureter and can cause recurrent infections of the

kidneys, frequently leading to permanent kidney damage. In a similar manner to stress urinary incontinence, it is possible to insert pellets or beads of silicone rubber or teflon in the submucosa of the bladder wall close to the ureteral orifice. Again, the procedure requires the permanent insertion of the implant.

Paediatric vesicoureteral reflux usually resolves itself as the bladder wall thickens. By the time a child is five years old the urinary system has usually matured sufficiently to make the implant material redundant. Again, it is possible for implant material to migrate from the implant site causing obstruction, occlusion or embolism at another site. Implants have also been associated with cancer, auto-immune and connective tissue disease.

The present invention provides a composition suitable for implantation in soft tissue (for example at or around a body orifice) in order to augment the volume of soft tissue. The composition of the present invention comprises particles of biodegradable glass in a suitable carrier medium. The carrier medium is required to ensure easy injection at the site of interest.

The currently available silicone, PTFE and collagen beads are all deformable. This property aids injection of the beads, but also contributes to their ability to migrate from the site of interest. By contrast, the glass particles of the present invention are non-deformable.

The composition is suitable for insertion in the bladder submucosa to treat stress urinary incontinence or vesioureteric reflux by bulking up the area around

the urethral sphincter or urethral orifice respectively.

Optionally, the glass particles dissolve over a relatively long period, typically one to five years, more usually one to two years.

Preferably, the glass particles are irregularly shaped. This contrasts to the commercially available implants which are formed from spherically shaped beads. The irregular shape of the glass particles encourages their encapsulation in fibrous tissue. Such encapsulation further reduces the rate of dissolution of the glass and also helps to prevent migration of the particles.

Typically, the glass particles used in the present invention may have a diameter of from 50 μm up to 2000 μm. More conveniently, however, the average diameter of the particles will be 1000 μm or less, usually 500 μm or less. Good results have been obtained with particles having an average diameter of 300 to 200 μm or less, for example 150 μm or less.

Particles having smaller diameters, e.g. 100 μm or less, particularly of approximately 50μm, or even less, are of especial interest.

One advantage of the present invention is that it is possible to form glass particles having such small diameters (e.g. 50-100 μm). Where such small particles are used the problems associated with injection are reduced. Additionally, once the particles have been located in the site of interest, the outside surfaces of the particles becomes tacky as the particles begin to dissolve into body fluids so that the particles become associated <u>in situ</u> in a sticky cohesive mass.

Such particle association greatly reduces the rate of particle migration and the health risks associated therewith. No such association has been observed with the prior art silicone, PTFE or collagen beads.

A carrier medium is generally used to assist injection of the particles. The carrier medium is typically glycerol, but other conventional carrier mediums (e.g. corn oil, sesame oil, sunflower oil or olibas oil) may also be used. A surfactant and/or suspending agent may also be included in the composition. Typical surfactants include, for example, benzyl benzoate, ethyl oleate and benzyl alcohol. Typical suspending agents include, for example, carboxymethylcellulose and alginate.

In a further aspect the present invention provides a method of augmenting an area of soft tissue in a body (e.g. thickening a wall of a body organ), said method comprising injecting a composition into the soft tissue (e.g. the submucosa of said wall), said composition comprising particles of a biodegradable glass.

Thus, the present invention provides a method of combatting vesicoureteric reflux by injecting a composition of the present invention into the bladder submucosa close to the ureteral orifice such that urine is substantially unable to pass up the ureter upon contraction of the bladder.

Likewise, if the composition of the present invention is injected into the submucosa in the vicinity of the urethral sphincter, stress urinary incontinence may be overcome due to the "bulking" effect of the injected particles.

The present invention may be used at other body areas where soft tissue augmentation has a beneficial effect. Examples include injection around the anal passage, in order to reduce blood flow at the site and hence combat development of haemorrhoids (piles). Likewise soft tissue augmentation may be beneficial to temporarily correct an "incompetent" cervix which would prevent sustainment of a pregnancy. The soft tissue augmentation of the present invention may further be used to build up portions of the body damaged by accident or surgery, allowing healing to take place. Particular mention may be made of reshaping the facial area of a patient. From the above examples it is clear that the composition of the present invention may be used not only to treat existing conditions but also for prophylactic and cosmetic purposes.

Generally the glass will be a controlled release glass (CRG). CRGs are vitreous inorganic polymers which dissolve over a pre-programmed period leaving virtually no residue. The components of manufacture are all present as natural body constituents hence CRGs show little or no cytotoxicity and exhibit a minimal tissue reaction.

The use of glasses which can dissolve in water and body fluid are well-known. These glasses are formed from phosphorus pentoxide and may be modified to dissolve over a period of months or years, as required. To date, such glasses have been used, in medicine, for the controlled release of a number of agents, for example, drugs, hormones and trace elements.

It is known that certain glasses, in which the usual glass former, silicon dioxide, of traditional glasses is replaced with phosphorus pentoxide as the glass

former, are soluble in water and body fluids. The rate of dissolution is controlled largely by the addition of glass modifiers such as calcium and magnesium oxide. In simple terms, the greater the concentration of the modifier the slower the rate of dissolution. The rates of dissolution which can be imparted to the glasses may range from minutes to months or even to several years. It is known to include in such compositions quantities of trace elements such as copper, cobalt and selenium which will be released from the glass as it slowly dissolves over the selected period of time.

The use of water-soluble glasses has been described for a variety of purposes in the literature. For example, UK Patent Specifications Nos 1,565,906, 2,079,152, 2,077,585 and 2,146,531 describe the gradual dissolution of the glasses as providing a means of controlled release of drugs, hormones, fungicides, insecticides, spermicides and other agents with which the glasses have been impregnated. The glasses are used, for example, in the form of an implant or bolus.

UK Patent Specification No 2,030,559 describes the use of selenium-impregnated water-soluble glass for providing controlled release of the selenium as a trace element into cattle and sheep, the glass being applied as a subcutaneous insert. UK Patent Specification No 2,037,735 also describes a subcutaneous implant of water-soluble glass, and in this case the glass is impregnated with copper; minor quantities of trace elements such as boron, arsenic, iodine, manganese, chromium, silver, gold and gallium may also be included.

Water-soluble glass has also been proposed for use in

prosthetics, for example in UK Patent Specification No 2,099,702, and for use in anticorrosive paints, as described in UK Patent Specification No 2,062,612. Further the literature provides for the use of such glasses in the controlled release of ferrous and ferric ions into the human or animal body by ingestion or implantation of the glass (UK Patent Specification No 2,081,703), and for the use of glasses in the controlled release of ions such as lithium, sodium, potassium, caesium, rubidium, polyphosphate, calcium and aluminium to patients by inclusion of the glass in a drip feed line (UK Patent Specification No 2,057,420).

Optionally the water-soluble glass may be a silver containing water-soluble glass. Advantageously the silver content may be introduced into the glass composition in the form of silver orthophosphate.

Suitable glasses include, for example, the ARGLAES™ glass of Giltech Limited.

The glass may be adapted by the use of glass modifiers to give a sustained release of silver ions over a set period.

In one embodiment the water-soluble glass comprises an alkali metal oxide M_2O, an alkaline earth oxide MO, phosphorus pentoxide P_2O_5 and silver oxide (Ag_2O) or silver orthophosphate (Ag_3PO_4).

Most preferably, said glass contains not more than 40 mole % M_2O or MO, not less than 10 mole % M_2O or MO, and not more than 50 mole % nor less than 38 mole % phosphorus pentoxide, optionally with the inclusion of

0.05 to 5.0 mole % silver oxide or orthophosphate.

Said alkali metal oxide may be sodium oxide (Na_2O), potassium (K_2O) or a mixture thereof; and said alkaline earth oxide may be calcium oxide (CaO), magnesium oxide (MgO), zinc oxide (ZnO) or a mixture thereof.

The glass may also contain less than 5 mole % silicon dioxide (SiO_2), boric oxide (B_2O_3), sulphate ion (SO_4^{2-}), a halide ion, copper oxide (CuO) or a mixture thereof.

Typically the soluble glasses used in this invention comprise phosphorus pentoxide (P_2O_5) as the principal glass-former, together with any one or more glass-modifying non-toxic materials such as sodium oxide (Na_2O), potassium oxide (K_2O), magnesium oxide (MgO), zinc oxide (ZnO) and calcium oxide (CaO). The rate at which the silver-release glass dissolves in fluids is determined by the glass composition, generally by the ratio of glass-modifier to glass-former and by the relative proportions of the glass-modifiers in the glass. By suitable adjustment of the glass composition, the dissolution rates in water at 38 °C ranging from substantially zero to 25 $mg/cm^2/hour$ or more can be designed. However, the most desirable dissolution rate R of the glass is between 0.01 and 2.0 $mg/cm^2/hour$. The water-soluble glass is preferably a phosphate glass, and the silver may advantageously be introduced during manufacture as silver orthophosphate (Ag_3PO_4). The content of silver and other constituents in the glass can vary in accordance with conditions of use and desired rates of release, the content of silver generally being up to 5 mole %. While we are following convention in describing the composition of the glass in terms of the

mole % of oxides, of halides and of sulphate ions, this is not intended to imply that such chemical species are present in the glass nor that they are used for the batch for the preparation of the glass.

The glass may be formed by a number of methods. It may simply be cast by conventional or centrifugal procedures, or it may be prepared via one or more stages of rod, fibre or tube drawing. Other preparation techniques include foamed glass. Following glass formation it will be comminuted into finely divided form.

Optionally, the composition of the present invention may contain an active ingredient. The term "active ingredient" is used herein to refer to any agent which affects the metabolism or any metabolic or cellular process of the patient (including growth factors and living cells), promotes healing, combats infection, hypergranulation or inflammation. Antibiotics and other anti-bacterial agents, steroids, painkillers etc are all suitable. Optionally, the active ingredient may be in delayed-release or controlled-release form.

The invention will now be further described with reference to the following, non-limiting, examples and Figures in which:

Fig. 1 H and E staining of 1240596-1 glass granule intramuscular (six months). Magnification x 125.

Fig. 2 H and E staining of 1240596-2 glass granule intramuscular (six months). Magnification x 125.

Fig. 3 H and E staining of 1240596-3 glass granule

intramuscular (six months). Magnification x 125.

Fig. 4 Neutrophil staining of muscle section containing implant 1240596-1. Magnification x 125 (black circles are air bubbles).

Fig. 5 Macrophage staining of muscle section containing implant 1240596-2. Magnification x 125.

Example 1

The CRGs will be implanted *in vivo* to assist in the evaluation of attenuation of the solution rate of the glass and to observe the acute tissue reaction at the submucosal implant site.

Materials

Two CRG compositions with slow solution rates (to be decided) will be prepared as rough granules 200-300μm in diameter. The granules will be suspended in glycerine BP, 8.5 ml glycerine to 10 g CRG. The suspensions will be packaged 2.5 ml in syringes. The syringes will be individually sealed in foil polyester/polyester pouches and sterilised by γ irradiation.

Method

The anterior bladder wall of the anaesthetised model (rabbit) is exposed and a small volume (0.5 ml) of CRG implant is injected into the submucosa on the left and right anterior bladder wall midway between the ureters and the neck of the bladder. The implant should create a small visible mound at the implant site. It is suggested that the CRGs used at the left and right

sites be of different solution rates, or that one of the sites contains an existing "control" implant material for comparison (eg MACROPLASTIQUE (Trade Mark) of Uroplasty, Inc ten animals would be required.

Evaluation

By placing the implants in the anterior bladder wall, it should be possible to look at the implant on a weekly basis using ultrasound. In addition, two animals would be sacrificed at two weeks, one month, six months and twelve months.

The ultrasound examinations should look at the implant material and any migration from the implant site should be reported. Acute fibrous capsule formation should be recorded. It may be possible to differentiate the CRG and its dissolution over the more prolonged terms.

On sacrifice, tissue reactions and acute inflammation should be recorded. Fibrous capsule development should be noted and presence of CRG (and glycerol in early stages) quantified for each implant site. Samples of surrounding tissues should be removed for histological examinations.

Results and Interpretations

An initial inflammatory response is anticipated at the implant site. It is hoped that a collagen capsule will form around the CRG granules. This capsule is expected to reduce the solution rate of the glass. It will be helpful to measure the attenuation of solution rate due to reduced fluid transport within the capsule. By one month surrounding tissue inflammations should have subsided and histology should show normal cell response. There should be no migration of the CRG

implant beads and the glycerol should be completely removed within the first two weeks.

In each sacrifice group there should be at least one "control" implant. The tissue response of the control should be compared with the CRG implant results.

Example 2

Materials and Methods

Controlled release glasses (CRGs) were formulated as follows:

	mole % concentrations		
	Na_2O	CaO	P_2O_5
I240596-1	5	48	47
I240596-2	15	38	47
I240596-3	25	28	47

A granular diameter range of 53-1000 μm was used for all CRGs.

0.1 g samples of the CRGs listed above were sterilised by dry heat (190°C for 3 hours) before implantation into black and white hooded Lister rats (Liverpool strain). Two samples were implanted into each animal. Three animals were employed at a time period of six months. The implants were placed bilaterally into a pocket created in the dorsa-lumbar muscle region of the animal. At the six month time of explantation, the implant and surrounding tissue was removed from the sacrificed animal and frozen immediately. The frozen sample was sectioned at 7 μm in a microtome cryostat. Analysis of the implant/tissue site was performed by staining the sample sections for various cytokines. A haematoxolin and eosin (H and E) stain was carried out

on each of the six retrieved samples, as well as neutrophil and macrophage staining. Immunohistochemical staining for ED1, ED2, CD4, CD8, Interleukin-1β (IL-1β), IL-2, Major Histocompatibility Complex (MHC) class II, α-β and Anti-β antigens have been completed. These stains allow the tissue response to the implant presence to be evaluated in the following manner:

H and E	Stains all viable cells and allows the tissue type and fibrous capsule to be easily identified by the characteristic structure shape of each tissue.
ED1	Recognises rat macrophages, monocytes and dendritic cells. Granulocytes are negative. The recognised antigen is predominantly located intracellularly, although some membrane expression occurs.
ED2	Recognises a membrane antigen on resident rat macrophages; monocytes, dendritic cells and granulocytes are negative. No other cell types but macrophages are positive for ED2, and it discriminates between thymic cortical (ED2+) and medullary macrophages (ED2-).
CD4	Expressed on most thymocytes and approximately two thirds of peripheral blood T cells. In humans and rats, CD4 is expressed on monocytes and macrophages. CD4 is an accessory molecule in the recognition of foreign

	antigens in association with MHC class II antigens by T cells.
CD8	Expressed on most thymocytes and approximately one third of peripheral blood T cells, which constitute the CD4 negative cells. CD8α is in all natural killer (NK) cells in the rat.
IL-1β	Expressed by B cells, macrophages and monocytes and its mRNA is present in a number of cells including T cells. In addition to activating T and B lymphocytes, interleukin-1 (IL-1) induces several haematological and metabolic changes typical of host response to infection and injury. IL-1 is an endogenous pyrogen, producing fever by its ability to increase hypothalamic prostoglandin. IL-1 also induces the release of several lymphokines, interferons and colony stimulating factors. With the exception of skin keratinocytes, some epithelial cells and certain cells in the central nervous system, mRNA coding for IL-1 is not observed in health in most other cells.
IL-2	More descriptively, T cell growth factor, has promise as an immune stimulant and an anti-tumour agent. IL-2 recognises activated rat T cells but not resting lymphocytes.

MHC Class II	Expressed by dendritic cells, B cells, monocytes, macrophages and some epithelial cells. Expression is increased by interferon α which also induces expression on fibroblasts, epithelial and endothelial cells.
α-β	Detects an α-β T cell receptor.
Anti-β	Directed at leucocytes. Also labels B cells among thoracic duct lymphocytes with little labelling in bone marrow and none on thymocytes. Acts as an isotope control.

Results and Discussion

The photographs in Figures 1-3 show H and E staining of the I2405961-3 implants respectively. As can be seen in these Figures, fibrous capsules have formed around each glass granule. Glass I240596-1 has the slowest solution rate as tested in-vitro, and this can be seen in Figure 1 also, as the sizes of the remaining glass granules in the rat muscle after six months are considerably larger compared to the other two glass compositions which both have faster solution rates (I240596-3 has the fastest solution rate in-vitro). The surrounding muscle tissue to the implant appears healthy. Figures 4 and 5 show photographs of neutrophil staining of implant section I240596-1 and macrophage staining of I240596-2 respectively. These photographs are typical of all the slides viewed, as all six sections contained insignificant neutrophil and

macrophage presence in the tissue. In the photograph of the neutrophil stained section, it can be seen that there are several mast cells near the implant site and throughout the tissue. This is expected in normal, healthy muscle tissue. The lack of macrophages and neutrophils indicates a lack of inflammatory response to the implant, showing that after a six month period, the glass granules appear to be accepted in-vivo.

The cytokine staining of the above antigens were all negative, correlating with the absence of neutrophils and macrophages in the tissue sections. Cytokines are regulatory peptides that can be produced by virtually every nucleated cell in the body, such as lymphocytes and monocytes. Cytokines are generally not constitutively produced, but are generated in emergencies to contend with challenges to the integrity of the host. Cytokines achieve these ends by mobilizing and activating a wide variety of target cells to grow, differentiate and perform their functions. This means that cytokines are key mediators of immunity and inflammation. The insignificant staining of the above indicates the acceptance of the glass implant into the body and shows that the glass presence is not inducing any inflammatory reaction in-vivo.

Conclusion

All the sections stained and viewed after the six month period showed healthy, normal muscle tissue containing a fibrous capsule coated glass granule. Staining of various cytokines gave a negative result, indicating the absence of inflammatory responses of the muscle tissue with the glass presence after six months.

Example 3

Soft Tissue Response to Glycerol Suspended Controlled Release Glass Particulates

This example investigated the soft tissue response of glasses with a range of particulate sizes of different dissolution rates, transported in a glycerol carrier.

Materials and Methods

The CRG was tested in particulate form of three different compositions and two different particulate sizes: X (200-300μm, 0.02 $mg/cm^2/hr$ solution rate), Y (200-300μm, 0.12 $mg/cm^2/hr$ solution rate) and Z (<53μm, 0.34 $mg/cm^2/hr$ solution rate), all suspended in glycerol. A control sample of glycerol only was also included in the experiment and was labelled sample W. Samples weighing 0.1 grams of each of the CRG's in glycerol and glycerol only were sterilised by gamma irradiation before implantation intramuscularly into Wistar rats. Two samples were implanted into each animal. Four animals at each time period of 2 days, 4 weeks, 9 weeks and 6 months were employed. The implants were placed bilaterally into a pocket created in the dorso-lumbar muscle of the animal. At the time of explantion, the implant and surrounding tissue was removed from the sacrificed animal and snap frozen. A microtome cryostat was used to cut 7μm thick serial sections. Analysis of the implant/tissue site was performed by specific staining the sample sections for various cell types. Neutrophils and macrophages were stained using enzyme histochemistry, ED1 (monocytes and immature macrophages), ED2 (mature tissue macrophages), CD4 (helper/inducer T-lymphocytes and macrophages), CD8 (suppressor/cytotoxic T-lymphocytes), interleukin-1β, IL-2 (activated T-lymphocytes), Major Histocompatibility Complex (MHC) class II (activated

macrophages and activated B-lymphocytes), α-β (T-lymphocytes) and CD45RA (B lymphocytes) antibodies have been used to immunohistochemically stain each sample.

Results and Discussion

Positive staining for neutrophils was observed after 2 day implantation with all of the materials. The neutrophils present were found in localised clusters near the implant site. However, neutrophils were not seen in the tissue sections of each of the implanted glasses or glycerol in the remaining time periods. Mast cells were present in all tissue samples, but it was noticed that an increased number of these cells were present in clusters near the implanted glass in sections containing glass X at 6 months, glass Y at 2 days and 6 months and glass Z at 4 weeks and 6 months. Enzyme staining and immunohistochemical staining both confirmed the presence of macrophages in all sections at all time periods except glass X at 6 months. The neutrophil presence at 2 days in all sections suggest an acute inflammatory response. The absence of these cells however in the remaining time periods indicate that this acute inflammation is quickly resolved. However, the presence of macrophages in all samples at all time periods except X at 6 months indicate an ongoing chronic inflammatory response to the presence of the implanted material. With glass X however, this chronic inflammatory response appears to have been resolved at 6 months. With one material, glass Z, tissues necrosis in association with the glass at 4 weeks and 9 weeks has been observed. This study demonstrates that particulate, degrading glass is stimulating an inflammatory response in soft tissue of time periods up to 6 months. It should be noted that very small particulate fast degrading glass is leading

to tissue necrosis and should be further considered for these applications. However, larger particulate, slower degrading materials are demonstrating effective potential for stress incontinence applications.

EXAMPLE 3

Inflammatory Response to Controlled Release Glass

Samples of a range of compositions of Controlled Release Glasses (CRGs) in granular form were analysed for the soft tissue response to determine their biocompatibility.

Materials and Methods

The CRG was tested in granular form (53-1000μm) of three different compositions: A (high in CaO, slow solution rate), B (medium solution rate) and C (low in CaO, fastest solution rate). Samples weighing 0.1 grams of each of the CRG's were sterilized by dry heat (3hrs, 190°C) before implantation into black and white hooded Lister rats. Two samples were implanted into each animal. Three animals were employed at each time period of 2 days, 1 week, 4 weeks, 8 weeks and 6 months. The implants were placed bilaterally into a pocket created in the dorso-lumbar muscle of the animal. At the time of explantion, the implant and surrounding tissue was removed from the sacrificed animal and snap frozen. The frozen sample was sectioned at 7μm thickness in a microtome cryostat. Analysis of the implant/tissue site was performed by using different staining techniques. Immunohistochemical staining using ED1 (monocytes and immature macrophages), ED2 (mature tissue macrophages), CD4 (helper/inducer T-lymphocytes and macrophages), CD8 (suppresser/cytotoxic T-lymphocytes), interleukin-1β, IL-2 (activated T-lymphocytes), Major

Histocompatibility Complex (MHC) class II (activated macrophages and activated T-lymphocytes), α-β (T-lymphocytes) and CD45RA (β-lymphocytes) antibodies have been performed. A haematoxylin and eosin (H and E) stain was carried out on each of the retrieved samples. Neutrophil and macrophage enzyme staining was also performed.

Results and Discussion

The tissue response to the range of CRG's can clearly be demonstrated as being different and dependant on the materials, involving neutrophils, macrophages and mast cells and not involving T or B lymphocytes.

Localised clusters of neutrophils were observed after 2 days implantation of each of the CRG's A, B and C. However, neutrophils were not seen in the tissue sections of each implanted glass in each of the remaining time periods.

Mast cells were scattered throughout all tissue sections as expected, but it was noticed that an increased number of these cells were present in clusters near the implant in sections containing CRG A at 9 weeks and 6 months, and in CRG C at 2 days, 9 weeks and 6 months.

The most predominant cell type in all sections was the macrophage confirmed by both enzyme staining and immunohistochemistry. Macrophages were observed in all of the sections for all of the time periods and were positive for ED1, ED2 and MHCII antibodies. The presence of neutrophils at 2 days in all three glass compositions indicate that an acute inflammatory response has occurred. The absence of the neutrophils

at all subsequent time periods suggest that the acute inflammatory phase had resolved. However, the observation of macrophages throughout all time periods up to and including 6 months indicates continued stimulus by the materials of a chronic inflammatory phase response.

CLAIMS

1. A composition suitable for implantation in soft tissue, said composition comprising particles of biodegradable glass in a carrier medium.

2. A composition as claimed in Claim 1 wherein the glass particles are irregularly shaped.

3. A composition as claimed in either one of Claims 1 and 2 wherein said particles have a diameter of 1000 μm or less.

4. A composition as claimed in any one of Claims 1 to 3 wherein said particles have a diameter of 300 μm or less.

5. A composition as claimed in any one of Claims 1 to 4 wherein said particles have a diameter of 50 μm to 100 μm.

6. A composition as claimed in any one of Claims 1 to 5 wherein said carrier medium is glycerol.

7. A composition as claimed in any one of Claims 1 to 6 wherein said carrier medium includes a surfactant and/or a suspending agent.

8. A composition as claimed in any one of Claims 1 to 7 comprising glass particles formed from a controlled release glass.

9. A composition as claimed in any one of Claims 1 to 8 comprising glass particles formed from a water-soluble glass.

10. A composition as claimed in any one of Claims 1 to 9 comprising glass particles formed from a silver containing glass.

11. Use of a composition as claimed in any one of Claims 1 to 10 for augmentation of soft tissue.

12. Use as claimed in Claim 11 wherein said soft tissue is the submucosa of the urethral sphincter.

13. A method of augmenting an area of soft tissue in a body, said method comprising injecting a composition as claimed in any one of Claims 1 to 10 into the soft tissue.

14. A method as claimed din Claim 13 which is used augment soft tissue for cosmetic purposes.

15. A method as claimed in Claim 13 wherein said soft tissue is the submucosa of a wall of a body organ.

16. A method of combatting vesicoureteric reflux by injecting a composition as claimed in any one of Claims 1 to 10 into the bladder submucosa close to the urethral orifice such that urine is unable to pass up the ureter upon contraction of the bladder.

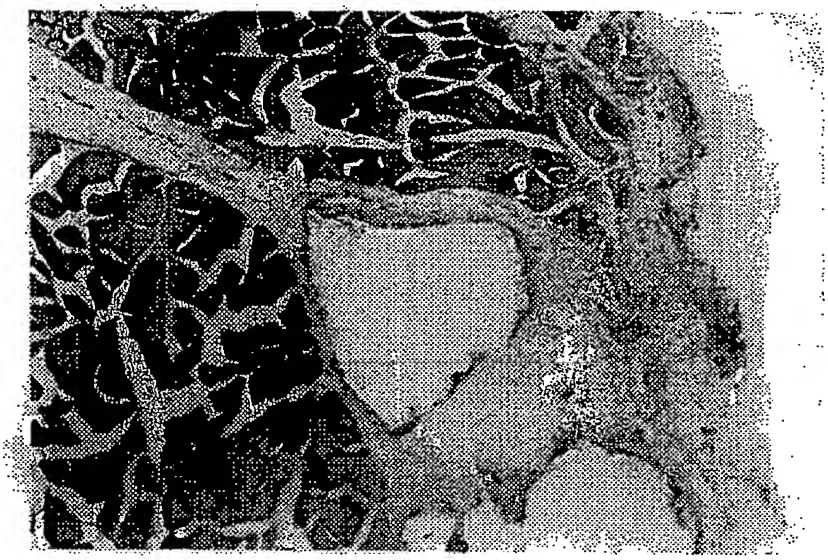

Fig. 1

Fig. 2

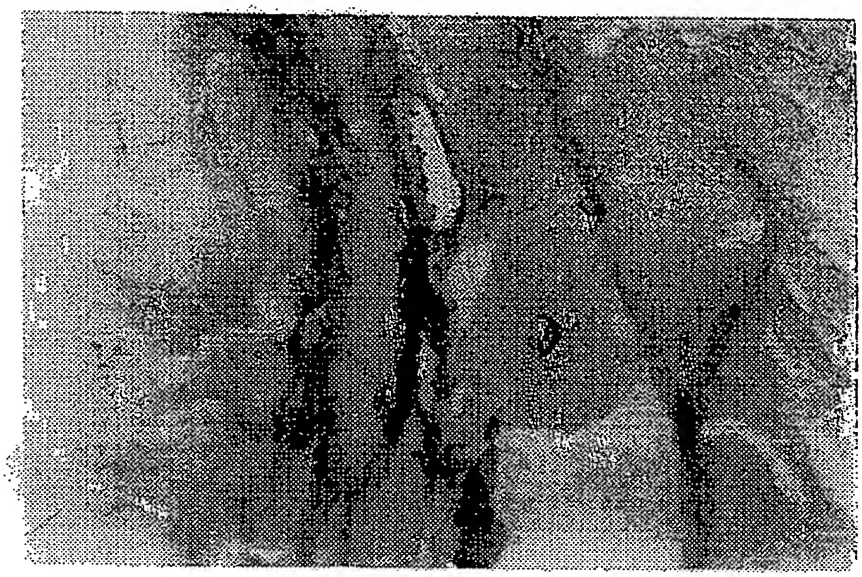

Fig. 3

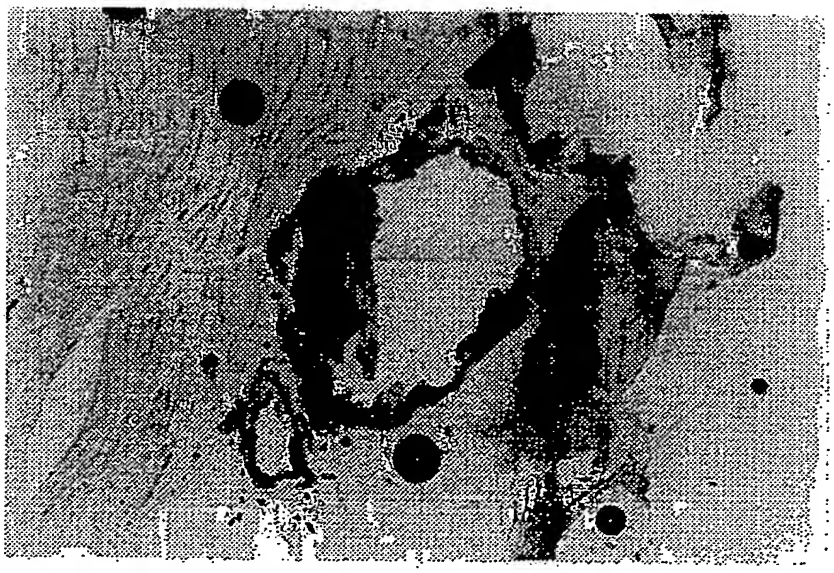

Fig. 4

Fig. 5

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 98/01017

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61L27/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61L C03C

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 204 382 A (WALLACE DONALD G ET AL) 20 April 1993 see column 7, line 36 - line 47; claims	1,3-6, 11-16
X	WO 93 16658 A (JSF CONSULTANTS LTD) 2 September 1993 see page 6, line 17 - line 28; claims	1,6,11, 12
Y	WO 93 15721 A (HUBBARD WILLIAM G) 19 August 1993 see claims	1-16
P,Y	WO 97 33632 A (GILTECH LTD ;GILCHRIST EILIDH (GB); GILCHRIST THOMAS (GB)) 18 September 1997 see claims; examples	1-16

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
4 August 1998	11/08/1998

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	ESPINOSA, M

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 98/01017

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	WO 91 17777 A (UNIV FLORIDA) 28 November 1991 see claims	1-16
A	WO 90 08470 A (GILTECH LTD) 9 August 1990	
A	WO 96 24364 A (GILTECH LTD ;HEALY DAVID MICHAEL (GB); GILCHRIST THOMAS (GB)) 15 August 1996	

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INTERNATIONAL SEARCH REPORT

.ernational application No.
PCT/GB 98/01017

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 13-16
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 13-16 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Int ational Application No
PCT/GB 98/01017

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INTERNATIONAL SEARCH REPORT

Information on patent family members

In ational Application No
PCT/GB 98/01017

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9624364 A		EP 0809506 A	03-12-1997